ELECTROPHORETIC PATTERNS OF FRESH, IRRADIATED-PASTEURIZED AND IRRADIATED-STERILIZED BEEF STORED AT 34° F.

by

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ELECTROPHORETIC PATTERNS OF FRESH, IRRADIATED-PASTEURIZED AND IRRADIATED-STERILIZED BEEF STORED AT 34° F.

INTRODUCTION

It is well known that proteins form the basic and underlying structures of all living organisms and are considered to be the most important constituent of muscle tissue even though meat contains 3.5 to 4 times more water than protein. In addition, proteins comprise the major part of the muscular enzyme systems, and these systems in turn exert considerable influence upon the continuity, properties and function of muscle in the live animal or in the post mortem state.

Shortly after death of an animal, the muscles pass into the so-called "rigor" state in which the muscles become hard, rigid and inextensible due to the shrinking and contraction of the muscle fibers. During rigor mortis, meat becomes extremely tough and is practically inedible. About 12 hours after the onset of rigor, the muscles begin to relax and undergo dissolution of rigor wherein the meat gradually increases in tenderness. Normally, beef is aged 5 to 10 days to accomodate increases in tenderness which are more or less proportional to the length of aging time.

Although the aging of meat results in the improvement of tenderness, the chemical events responsible and accompanying this

change have not been thoroughly elucidated. Relaxation of the muscle or the resolution of rigor mortis through the activity of microorganisms, bacterial proteolytic and catheptic enzymes appear to be the main factors involved in this phenomenon.

The precise function of catheptic enzymes in the tenderization of meat during the aging period is not well defined since large populations of proteolytic microorganisms are also generally found in meat being aged. In most of the work thus far reported in the literature, proteolysis has been studied by measuring the accumulation of amino acids. However, proteins can undergo considerable degradation before many amino acids are liberated and detected. The technique of electrophoresis, extensively reviewed by Block et al (10, p. 333-409), provides a method of studying the proteolytic changes occurring in meat proteins during the entire aging or tenderization process. Since it is necessary to study the initial proteolytic changes occurring in the proteins to determine whether proteolysis is of much importance in the tenderization of meat, the effects of contaminating microorganisms must be minimized or eliminated. The population of microorganisms can be greatly reduced or completely eradicated by subjecting the meat to ionizing irradiations. Furthermore, irradiation provides a means of pasteurizing and sterilizing meat while supposedly leaving the catheptic enzymes fairly well intact (17, p. 61;

20, p. 23).

The study presented in this thesis pertains to the effects of the inherent muscle enzymes upon the degradation of beef muscle proteins during the tenderization or aging period. More specifically, changes in the electrophoretic patterns of raw, irradiated-pasteurized and irradiated-sterilized beef samples were systematically determined over a prolonged storage period to achieve the above objective.

LITERATURE REVIEW

After the resolution of rigor mortis, muscle tissues undergo a process of "autolysis" or "proteolysis." Both of these terms are used synonymously in reference to the extensive breakdown or degradation of muscle proteins to peptides and amino acids by the action of tissue proteinases. These proteolytic enzymes are specifically known as cathepsins in order to distinguish them from the proteolytic enzymes of the digestive tract (66, p. 35).

Muscle Proteins

The muscle proteins are generally grouped into four different fractions on the basis of their extractability, locality in the muscle cell, and physiological properties. These groups are listed as follows: the sarcoplasmic proteins, granular proteins, stroma proteins, and myofibrillar proteins (64, p. 1-3).

1. The Sarcoplasmic Proteins

The sarcoplasmic proteins are often called the "soluble proteins" because they are readily extracted from the muscle with water or with neutral salt solutions of low ionic strength ($\Gamma/2 < 0.2$). These proteins are found in the space between the myofilrils. Myofibrils, various phosphokinases, and all of the

enzymes of the glycolytic system are the major constituents of this group. The sarcoplasmic proteins do not appear to be involved in the filamentous organization of muscle or in the structural reorganization that results in contraction. The function of these proteins appears to be mainly concerned with the metabolic activities of the muscle cells.

2. The Granular Proteins

The proteins of the granules and the sarcoplasmic proteins are both extractable from muscle homogenates by solvents of low ionic strength. The granular proteins can then be separated from the soluble proteins by differential centrifugation. Nuclei, mitochondria and the microsomes are the major components of this fraction. These components are mainly involved in the oxidative activities of the muscles. The granular proteins are localized between the myofibrils and somewhat in the specialized Z membranes. Although these proteins do not appear necessary for muscle contraction, some of their components or products may indirectly effect the behavior of the myofibrillar proteins which are directly responsible for muscle contraction.

3. The Stroma Proteins

These proteins are found in the residue after the extended extraction of muscle tissue homogenates with strong salt solutions.

The stroma proteins are of a collagenous nature and probably are involved in the make-up of various muscular membranes. Research on the stroma proteins has been neglected because of their poor solubility properties. Thus, the knowledge of these proteins is quite limited.

4. The Myofibrillar Proteins

These proteins are directly involved in muscle contraction and provide for the filamentous organization of muscle. The myofibrillar proteins are commonly known as the "structural proteins" or "insoluble proteins" of muscle and are extracted with neutral salt solutions of high ionic strength ($\Gamma/2 > 0.5$). Their fibrous nature is shown by the high viscosity of the extract when these proteins are taken into solution. Myosin, actin and tropomyosin are the major components of this group. The myofibrils which make up the contractile structure of muscle contain a large percentage of the myofibrillar proteins.

The above classification of muscle proteins was devised by Szent-Gyorgyi (64, p. 1-3), one of the foremost authorities on muscle. However, in much of the work reported in the literature on the proteolysis and aging of meat, the muscle proteins have been considered on the basis of the following three groups: myogen proteins, muscle plasma proteins, and stroma proteins

(68, p. 307). Under this classification, the myogen proteins includes both water soluble groups (sarcoplasmic and granular proteins) of Szent-Gyorgyi (64, p. 1-3), while the muscle plasma proteins are identical to the myofibrillar proteins. The stroma proteins are synonymous in both classifications.

Catheptic Enzymes

The intracellular proteinases that promote autolysis are now classified as cathepsins, but originally the term "cathepsin" was used to denote what was thought to be a single proteinases of animal tissues. Although beef muscle contains several catheptic enzymes, the exact number involved in proteolysis is not known (66, p. 35). However, three separate proteinases of muscle have been characterized thus far. These have been designated as cathepsin A, cathepsin B, and cathepsin C (23, p. 700).

Research results on the catheptic enzymes are quite limited due to the extreme difficulties encountered in the extraction, isolation, purification and characterization of these enzymes. None of the cathepsins has been prepared in the crystalline form. Studies on the properties of these enzymes have been carried out by the use of simple synthetic substrates of known structure to gain the present available knowledge (23, p. 700).

Balls (3, p. 85) observed a cathepsin that was still active

in frozen beef muscle which had a pH optimum around 4.1. Martin (34, p. 260), Nardone (43, p. 750), Noke and Neurath (59, 127-135), and Zender et al. (68, p. 309-313) have reported on some of the catheptic activity in a variety of tissues from several species of animals. Fruton et al. (22, p. 763) found that beef spleen contains at least 4 cathepsins as determined on the basis of specificity for synthetic substrates.

Fruton and Simmonds (23, p. 702) state that the present listing of the intracellular proteolytic enzymes of muscle is not a complete one since the specificity for many have not been adequately characterized. Furthermore, Whitaker (66, p. 36) has emphasized the obscurity of the role of the proteolytic enzymes in the autolysis of meat.

Proteolysis and Aging of Meat

Much of the work pertaining to proteolysis in meat has been obtained by following the changes in the various nitrogen fractions during the aging period. As has been previously state, considerable proteolysis may occur before it can be detected by noting the changes in the nitrogen components. Furthermore, many of the reported changes have been observed in meat held appreciably longer than the normal aging period.

In some of the earliest work on the storage of beef, Hoagland et al. (27, p. 15-65) reported that the noncoagulable

nitrogen rises progressively as time proceeds. This increase was also observed by McCarthy and King (36, p. 295-299) at a later date. According to Bate-Smith's (6, p. 29-30) interpretation of the data of Hoagland et al. the noncoagulable nitrogen represented about 12 per cent of the total nitrogen. Therefore, an increase of 8 per cent in this content, as occurred between the first and 30th day of storage, would indicate that only 1 per cent of the protein present was hydrolyzed to soluble end products. On this basis, after 180 days of storage only 6 per cent of the protein would be degraded to the soluble level. Bate-Smith (6, p. 30) concludes that the tenderness changes have to be explained in terms of much less drastic proteolysis than is shown by the complete breakdown to noncoagulable end products.

Aging of meats consists of the transformation of albuminoids of the muscle into coagulated substances, according to Smorodintsev and Nikolaeva (58, p. 5947). Sadikov and Shodhin (54, p.6466) conducted preliminary studies on the proteolytic enzymes of meat and reported that a slight alteration of globulins occurred after 2 days aging at 17° C. while marked changes were observed after 27 days storage. They also noted extensive peptone formation in the meat stored at 17° C. for four months. This breakdown proceeded to amino acids in a few days when storage temperature was maintained between 55-60° C.

Husaini et al. (29, p. 366-369) and Wierbicki et al.

(67, p. 506-511) have reported that there was no change in the non-protein nitrogen content of beef aged up to two weeks. Hodgkiss and Jones (28, p. 4-5) and Shewan and Jones (57, p. 491-498) have stated that with the exception of alanine and lysine which decreased slightly and glutamic acid which increased 300 per cent, the amino acids resulting from protein breakdown remained constant in fish stored at 0° C.

A number of other workers, however, have found changes in the non-protein nitrogen during the aging or storage of beef. In studies on the aging of beef, Ginger et al. (24, p. 410-416) noted that the amino nitrogen content of the non-protein nitrogen fraction increased with storage time. They also found increased amounts of arginine, leucine and tyrosine in the exudate and non-protein nitrogen fractions of beef aged at 36° F. for 2 weeks. They attributed these results as being indicative of proteolytic enzyme activity occurring during the aging of beef.

Olson and Whitehead (46, p. 180-183) observed a 41 per cent increase in total soluble nitrogen and a 14 per cent increase in non-protein nitrogen in beef muscle after 29 days of aging at 34° F. Colombo and Gewasini (13, p. 14137) and Niewiarowicz (45, p. 9470) have reported a progressive increase in the free amino acid content of beef on aging for 6 to 18 days at 0° to 4° C. They found the greatest change in the content of alanine, cystine,

leucine and glutamic acid. Antoniami and Monzini (2, p. 4006) and Monzini (38, p. 4143) have published results of their studies on proteolysis which show that the amino acid nitrogen content of beef increased by 5 to 6 per cent of the total nitrogen when stored at temperatures of -25 to -40° C. for 10 months. However, Monzini and Lissoni (39, p. 4420) later reported that the amino acid content decreased on storage at -25 to -40° C. for 48 months. A decrease in the glycine soluble proteins and a corresponding increase in amino acid content of rabbit and lamb muscle stored under aseptic conditions at 25° C. for one month has been observed by Zender et al. (68, p. 309-313).

Proteolysis has been reported in beef and chicken stored in the frozen condition, according to the reports of Hiner et al. (26, p. 223-229), and Swanson and Sloan (63, p. 643-649). These workers found over-all increases in non-protein nitrogen, water soluble nitrogen and amino nitrogen with storage time.

Effect of Ionizing Irradiation on Proteins

Proteins may be altered in various ways by ionizing irradiations. Aggregation, fragmentation and denaturation are the major structural modifications observed in irradiated proteins (41, p. 424). Proteins are condensation polymers composed of many amino acids linked together by peptide bonds. Hence, the nature of irradiation effects upon proteins is dependent upon the polymer

chain length, amino acid composition, and the chronological sequence of the amino acids. During irradiation, a protein molecule may act as a single molecular component, as occurs upon denaturation, or as a composite of several amino acids, with each acid exhibiting a specific irradiation sensitivity (19, p. 156-158).

Hannan (25, p. 192) has described the physical changes in irradiated proteins as that of some form of denaturation with the character of changes varying with the nature of the free polymer and the irradiation conditions. However, he concluded that it is most likely that the primary valency bonds are broken and reactive free radical fragments are formed.

McArdle and Desrosier (35, p. 527-532) observed significant changes in the protein structures of casein and egg albumen after irradiation. Although the pattern of changes differed in these two proteins, the build up in free sulfhydryl groups definitely indicated that the sulfur linkages and hydrogen bonds were attacked and caused molecular rearrangement. Since they did not observe an increase in amino nitrogen with these molecular changes, they assumed that there was very little attack upon the peptide linkages.

McArdle and Desrosier (35, p. 527-532) also compared the electrophoretic patterns of non-irradiated solutions of casein with those irradiated at a dosage level of 1.5×10^6 rep. They

reported that the irradiated casein produced an entirely new electrophoretic component differing distinctly in pattern and mobility from the alpha and beta components of the non-irradiated casein. They described the changes in the irradiated casein as those of molecular splitting followed by polymerization of the fractions to cause an increase in viscosity. This pattern of modification resembles the description of the heat denaturation of casein as reported by Neurath and Bailey (44, p. 280) in which the molecules split, polymerize, and finally coagulate.

Molecules of egg albumen do not appear to be split by irradiation (35, p. 527-532). Instead, it appears that the globular molecules change slowly thereby resulting in the formation of an asymmetrical molecule which results in increased viscosity. The molecular rearrangement progressed linearly with radiation dosage. Thus, it seemed that polymerization had not occurred. Hence, the development of an asymmetrical molecule from the globular state by transformation appears to be responsible for this molecular change.

McArdle and Desrosier (35, p. 527-532) reported that the electrophoretic pattern of irradiated egg albumen produced a single component that was definitely different in pattern and mobility from any of the components observed in the non-irradiated albumen.

The heat denaturation of egg albumen compares closely with that caused by irradiation. Neurath and Bailey (44, p. 830) state that the heat denaturation of egg albumen causes the globular molecules to change and give asymmetrical forms which then polymerize and coagulate.

Doty et al. (18, p. 424) found an increase in the non-protein nitrogen compounds in ground lean beef after irradiation which was accompanied by an appreciable decrease in the soluble protein. In addition, they noted an increase in the formation of methyl mercaptans and hydrogen sulfide. Zender et al. (69, p. 390) have also reported a decrease in the glycine soluble protein content of beef muscle after irradiation.

Although the electrophoretic pattern of lyophylized blood plasma irradiated at a level of 1×10^6 rep was no different from that of the control plasma, the pattern for plasma radiated at 2×10^6 rep was found to be considerably different from that which was non-irradiated or received a low dosage (8, p. 266). Batzer and Doty (7, p. 64) recorded an increase in carbonyl compounds and pH which corresponded to increases in irradiation dosage. Other investigators (17, p. 61-63; 41, p. 425) have also found that the amount of protein breakdown is related to the level of irradiation dosage.

Effect of Ionizing Irradiation on Amino Acids

The irradiation of amino acids was first studied by Stenstrom and Lohmann (61, p. 673; 62, p. 755). They investigated the effects of X-ray irradiation upon the stability of aqueous solutions of tyrosine, tryptophane and cysteine. Stenstrom and Lohmann found that the above amino acids were decomposed by irradiation and that the dilute solutions appeared to be more affected by a given dose than were the more concentrated solutions.

Morgan (41, p. 426) has stated that the amino acids containing sulfur (methionine, cystine and cysteine) and the ring-containing amino acids (histidine, hydroxyproline, phenylalanine, proline and tryptophane) are extremely sensitive to irradiation.

The characteristic effect of ionizing irradiation on the amino acids is one of deamination (30, p. 7-10; 51, p. 535-538). Principal deamination products of amino acids are ammonia and the corresponding aldehyde. The mechanism of ammonia liberation is not clear but it appears to involve direct deamination resulting from attack by H· and ·OH radicals. A radical formed by removal of the NH_2 group in the deamination process allows for subsequent recations of the radical to form a variety of oxidative products (19, p. 156-158). According to Dale and Davies (15, p. 129-134), the liberation of hydrogen sulfide or the oxidation of the S-H groups to disulfide and finally sulfinic - SO_2H and sulfonic -

SO_3H acid groupings occurred during the irradiation of the sulfur-containing amino acids.

Proctor and Bhatia (51, p. 535-540) found that the hydroxylation of the ring in aqueous solutions of cylic amino acids occurred in some of their experiments. The hydroxylation of phenylalanine to tyrosine and a second hydroxylation forming 3,4-dihydroxy phenylalanine provided evidence for their conclusion.

Irradiation levels of 2×10^6 rep produced no significant destruction of the amino acids in milk, turkey and beef according to Sheffner et al. (56, p. 455-461). Metta and Johnson (37, p. 479-490) reported no change in the biological value of beef following irradiation at 3×10^6 rep. Proctor and Bhatia (50, p. 357-361) have stated that irradiation caused no significant destruction of any of the ten amino acids in fish.

Effect of Ionizing Irradiation on Enzymes

The influence of ionizing irradiations upon the enzyme systems has been the subject of numerous investigations. The observation that proteolysis occurred during the storage of bacteriologically sterile meats has been reported by several workers (20, p. 23; 31, p. 346-352; 32, p. 193-195; 49, p. 496-499). Doty and Wachter (17, p. 61-63) have mentioned that there was very little destruction of proteinase in beef irradiated at

0.5×10^6 rep, but at a higher dosage level, 1.6×10^6 rep, there was about a 50% loss in the apparent activity in some samples. The formation of tyrosine crystals during storage of irradiated-sterilized raw meat led Pratt and Ecklund (49, p. 496-499) to conclude that proteolysis of a general nature had occurred. Drake and Giffee (20, p. 23) noted extensive tyrosine formation in radiated pork stored at 100° F. for 3 months. They also reported an increase in the free amino acid content in the press fluid of irradiated pork stored at 72° F.

Dale (14, p. 1367), Dunn et al. (21, p. 605) and Tytell and Kersten (65, p. 521-525) have indicated that a large amount of irradiation, possibly ten times as great as that necessary for bacteriological sterilization was required to destroy or inactivate the enzymes in various foods. Proctor and Goldlith (53, p. 376-379) observed some peroxidase activity in milk irradiated at 10,000,000 rep. O'Meara (47, p. 19-23) has stated that the radiation resistance of specific enzymes in various foods is not always the same since the amount of moisture present exerts considerable influence.

Dale (14, p. 1367) postulated that the enzyme molecules were not directly affected by ionizing radiation. Instead, the protein moiety of the enzyme or the prosthetic groups received the blunt of the irradiation. When acting upon the protein moiety,

irradiation may destroy certain selective groups in the side chain which are essential for enzyme activity or it may rupture the hydrogen bonds and thus cause precipitation.

Pollard (48, p. 99-100) and Setlow (55, p. 471-483) have described the rate of enzyme destruction as that of an exponential decrease in activity with increasing irradiation dosage.

Enzymes irradiated in the dry state were found to be inactivated by excitation (48, p. 99-109) while those in solution were inactivated indirectly by OH and OH_2 radicals in the solvent (4, p. 109-121). The H_2O_2 appeared to be of minute influence (5, p. 188-201).

In their study of the irradiation effects on the activity of trypsin, Bier and Nord (9, p. 204-215) noted that this enzyme was very resistant to irradiation *in vitro*. Furthermore, they reported that irradiation sufficient to sterilize many food substances failed to inactivate dilute aqueous trypsin solutions.

From the preceding discussion it is evident that enzymes are considerable more resistant to irradiation than are the microorganisms. Conversely, microorganisms are less susceptible to heat treatment than are the enzymes. Drake and Giffee (20, p. 23) found that the enzymes responsible for proteolysis in meat are inactivated by heating to 160° F. and holding for 10 minutes. However, the extensive studies on temperature inhibition of enzymes recently reported by Chiambalero *et al.* (12, p. 782-784)

indicate that the proteolytic enzymes of irradiated beef are inactivated in 1½ minutes when the internal temperature of the meat reaches 160° F.

Effects of Ionizing Irradiation on Microorganisms

According to Morgan (41, p. 423-427), irradiation doses of 500,000 rads and 4.8×10^6 rads have been established as the dosage levels required for pasteurization and sterilization, respectively. Morgan describes irradiation-pasteurization as low level irradiation which destroys about 98 per cent of the microorganisms. High level irradiation or sterilization is considered as that dosage which destroys all spore-forming food spoilage organisms as well as all food poisoning organisms (42, p. 357-366).

Different microorganisms exhibit widely varying degrees of irradiation sensitivity. Brownell et al. (11, p. 55-57) irradiated a wide variety of bacteria, mold, yeasts, and viruses at various dosage levels and found that the spore forming bacteria were the most radiation resistant. Hannan (25, p. 49) reported that the more rapidly developing cells and vegetative bacteria were more irradiation sensitive than bacterial spores.

Numerous investigators (11, p. 55-57; 16, p. 44-50; 40, p. 24-28; 42, p. 357-366) have reported that Clostridium botulinum is

one of the most radiation resistant organisms known. In his review on radiation preservation of foods, Morgan (40, p. 24) has stated that a dosage level of 4.8 megarads is necessary to destroy the spores of Cl. botulinum. Furthermore, Denny et al. (16, p. 46-50) found that this organism was more resistant to irradiation in natural foods than in neutral phosphate when radiated at temperatures below 32° F.

Anderson et al. (1, p. 575-578) have reported on the isolation of a radio-resistant micrococcus from ground beef and pork that had been irradiated at 3.0×10^6 rep. They also noted that this organism was resistant to radiation dosages as high as 6.0×10^6 rep.

Lea (33, p. 71) theorizes that direct hits at or near the sensitive parts of the organisms caused the destruction of bacteria. However, Proctor and Goldlith (53, p. 376-380) observed that the indirect effects of radiation upon the medium aided in the destruction of Staphylococcus aureus. The latter workers also stated that St. aureus withstood irradiation better in complex media than in simple ones.

Bellamy et al. (8, p. 266-269) reported that a radiation level of 75×10^3 rep was sufficient to kill all of the pseudomonads responsible for spoilage in fresh meat if the level of contamination was moderate. There are several reports found in the

literature giving a wide range of dosage levels necessary for the sterilization of meat. These levels range from 1.5×10^6 to 5.0×10^6 rads. However, in view of the resistance and toxicity of Cl. botulinum, a minimum dosage level of 4.8×10^6 rads, should be employed for the sterilization of meat.

EXPERIMENTAL PROCEDURE

Source of Raw Material

An intact loin or rib eye muscle (longissimus dorsi) was taken from a cow carcass of utility grade at a local meat packing plant three hours after slaughter. After the muscle was rigidly trimmed of all external fat and membrane, samples of lean tissue weighing approximately 50 grams were placed in saran-mylar-polyethelene pouches and permanently closed by heat sealing. Also at this time, a control sample was removed and immediately prepared for electrophoretic and spectrophotometric analysis. The sealed pouches of beef were then sealed in metal cans to protect them from being torn during subsequent handling in irradiation and thereby exposing the contents to contaminating atmospheric conditions.

Irradiation

The packaged beef samples were exposed to gamma irradiation in the Co_{60} irradiator facility located in the Pilot Plant of the Food Technology Building, Oregon State University. Samples irradiated at the pasteurization level received a total dosage of 1.0 megarad while those subjected to sterilization treatment received an irradiation dosage of 4.8 megarads. Throughout the irradiation

process, all samples were held in the high flux chamber with the average dose rate being 274,350 tissue rads per hour. On this basis, 17.5 hours were required for samples receiving the sterilization dosage of 4.8 megarads while 3.67 hours were needed for samples subjected to pasteurization at the 1 megarad level. During the irradiation-pasteurization period, the samples were repositioned in the chamber at time intervals of 70 minutes in order to insure that all samples were subjected to uniform radiation. Samples subjected to irradiation-sterilization were repositioned after 6 and 12 hours of radiation.

During the entire irradiation operation, temperature of the high flux chamber was maintained within a range of 42-46° F. by the continuous circulation of ice water through the outer chamber shell.

Storage

Each individual pouch or sample, regardless of treatment, was sealed in a #2½ "C" enamel can and stored at 34° F. During the storage period, non-irradiated or control samples were analyzed at 1 day intervals for 19 days, then at 3 day intervals up to and including 52 days. The pasteurized beef was analyzed at 3 day intervals during the first 18 days of storage and every 5th day

thereafter up to and including 88 days. Sterilized samples were analyzed after 15, 35, 55 and 75 days of storage. In addition, samples were analyzed immediately after completion of the sterilization and pasteurization treatments.

Sample Preparation

The procedure of Zender et al. (68, p. 308) was used for the glycine extraction of the muscle proteins. Beef muscle was homogenized with three times its weight of 0.2 M glycine NaOH buffer, pH 8.6, in a Waring blender at 32° F. The glycine buffer solubilized both the water-soluble myogen proteins and part of the myofibrillar proteins equivalent to 40% of the total fresh muscle proteins (69, p. 385). This extracting buffer has a low specific electrical conductivity which permits its direct use in electrophoresis measurements. After homogenization, the resulting suspensions were centrifuged at 32,500 times gravity for 30 minutes at 32° F. in a Servall refrigerated centrifuge. The supernatant was then filtered through Whatman #1 filter paper. The filtrate contains all aqua-soluble components of muscle and a good part of fibrillar proteins (68, p. 308).

Approximately 50 milliliters of the filtrate were dialyzed at 34° F. for 48 hours against 4 liters of veronal buffer having a pH of 8.6 and an ionic strength of 0.05. The dialysis bag was

then placed before a fan at 34° F. and the solution was allowed to evaporate until approximately 10 milliliters remained. Hence, the solution was thus concentrated to contain sufficient protein (1 to 2 grams per cent) for electrophoretic analysis.

Electrophoretic Analysis

The electrophoretic runs were conducted with a Spinco Model R paper electrophoresis unit according to the procedure outlined in the Spinco Manual (61, p. 18-20). A veronal buffer having a pH of 8.6 and an ionic strength of 0.05 was used for all samples. The extracts were applied at the middle of the Whatman #3 paper strips at a rate of 0.01 to 0.03 milliliter per strip, depending on the protein levels of the concentrated extracts. All electrophoretic runs were made at a constant current of 10 milliamperes (ma.)for 17 hours at 4° C. After completion of a run, the strips were dried in an oven for 30 minutes at 110-120° C. The strips were then dyed with bromphenol blue and scanned with a Spinco Analytrol to determine the electrophoretic pattern of each sample.

Tyrosine-Tryptophane Index

Aliquots of the original extracts, after centrifugation and filtration, were diluted and the optical density measured at 279 m in a Beckman DU spectrophotometer both before and after

precipitation of proteins with 10 per cent trichloracetic acid (TCA). The optical density of the extract prior to TCA precipitation measures the level of glycine soluble proteins (GSP). After the extract is precipitated with TCA, the optical density is indicative of the concentration of the glycine soluble amino acids (GSAA). Zender et al. (68, p. 308) have found that a wave length of 279 m is characteristic for tyrosine-tryptophane either free or bound to proteins. They compared variations in the 279 m index with fluctuations in either protein or non-protein nitrogen concentrations and found that the variations corresponded well one to the other. Although this index is only a relative measure of protide concentration it is quite useful for estimating the level of proteolysis during storage.

RESULTS

Electrophoretic Data

The paper electrophoresis technique produces results that are primarily qualitative. Because of electrostatic, osmotic and diffusion effects, buffer flow and other factors, it is difficult to obtain quantitative results comparable to those obtained by the use of the moving boundary electrophoretic technique.

Although many electrophoretic patterns were obtained during the course of this study, only the representative diagrams showing the significant changes which occurred in the control and irradiated meats during the storage period are presented. The areas and mobilities as discussed for the subsequent diagrams, although relative, are comparable from one treatment to another. The original electrophoretic strips are shown at the top of each of the corresponding figures. These strips were included to provide visual evidence of the separation of the various protide fractions.

1. Non-Irradiated or Control Samples

The electrophoretic pattern of the control sample is given in Figure 1. The protein was extracted for this sample approximately 4 hours post mortem. Four distinct peaks, designated as

A, B, C and D, are shown which indicates four different protein fractions, each having a different rate of mobility.

Figure 2 shows the diagram for the non-irradiated beef after 3 days of storage at 34° F. During this brief aging period, considerable proteolysis had occurred. A new protide fraction, D_1, has appeared as well as some relative changes in the original fractions. There appears to be increases in the A, B and D fractions while a slight decrease seems apparent in fraction C.

After 10 days storage, the control beef underwent further modification as shown in Figure 3. The appearance of another new fraction, B_1, can be readily observed. Other noticeable changes indicate that fractions A and B may have increased slightly.

The diagram presented in Figure 4 is that of the control meat stored for 18 days. It appears that the meat underwent considerable proteolysis between the 10th and 18th days of storage. Fraction A appears to have declined slightly while the B_1 and D_1 fractions have shown considerable increases. Fraction D has shown an appreciable reduction while the B and C components have remained fairly constant.

In Figure 5, the electrophoretic pattern for the non-treated meat after 49 days of aging is illustrated. This diagram shows that fractions A and B_1 have declined somewhat radically from their heights shown in the previous pattern, Figure 4. The curve for component A appears to resemble that of fraction A obtained in

the initial pattern, Figure 1. Component D_1 appears to have increased slightly between the 18th and 49th days of storage while the other fractions remained stable.

2. Irradiated-Pasteurized Samples

The control electrophoretic pattern for the irradiated-pasteurized samples is presented in Figure 6. This sample was non-irradiated meat aged 24 hours at 34° F. Due to the small capacity of the irradiation chamber, a 24 hour delay occurred before samples to be pasteurized could be irradiated at a dosage level of 1.0 megarad. For this reason a separate control sample aged for 24 hours was necessary. The four fractions shown in this diagram are identical to those given in Figure 1, the initial sample.

Figure 7 contains the diagram for the pasteurized meat stored for 9 days at 34° F. A new component, B_1, appears in this pattern. In the non-irradiated samples, fraction D_1 did not appear until after 10 days of storage, whereas the first new fraction appearing in the control beef was D_1 which appeared after 3 days of storage. Figure 7 also shows that fraction B increased during the first 9 days of storage while the other components showed practically no change.

In Figure 8, another new component, D_1, appears in the

pasteurized beef after 12 days of storage. This pattern also shows that there was a tendency for some increase in the relative concentrations of fractions B, C and D.

The pattern for the pasteurized beef after being stored for 88 days is given in Figure 9. During the interval between the 12th and 88th days of storage, the new fractions, B_1 and D_1, increased to the extent that they over-shadowed the original fractions of B and D. Figure 9 also shows that fraction A may have decreased slightly while component C did not change. It is of interest to note that the pattern in Figure 4, control beef after 18 days of storage, and the diagram in Figure 9, pasteurized beef stored 88 days, tend to show similarities in the mobilities and concentrations of the six protide components.

3. Irradiated-Sterilized Samples

The electrophoretic pattern of the sterilized beef obtained immediately after irradiation at a dosage level of 4.8 megarads is shown in Figure 10. The sterilized beef apparently had not been modified to any noticeable extent by the irradiation treatment. This supposition is based on the similarity between the electrophoretic pattern shown in Figure 1, the initial control, and that of Figure 10, the sterilized sample.

Figure 11 contains the pattern of sterilized beef after 75

days of storage at 34° F. During this 75 days storage period, it appears that some modification may have occurred as indicated by changes in the relative area of each component. However, no new fractions or components were noted. There is also an indication that there may have been some denaturation occurring because of the poor migration of the glycine extract from the point of application on the paper strip. The extremely dark zone on the center of the paper strip, as shown at the top of Figure 11, provides the basis for this statement.

Tyrosine-Tryptophane Index

Although the tyrosine-tryptophane index must be considered as a relative measure of the protide concentration, it does provide evidence which helps to support the electrophoretic data on the pattern of proteolytic changes occurring during the storage of meat.

1. Glycine Soluble Proteins

Results of the glycine soluble protein (GSP) concentrations of the samples of the control and irradiated treatments plotted against storage time are given in Figure 12 and also are tabulated in Table 1.

The tyrosine-tryptophane (TT) index for the glycine soluble proteins of the non-irradiated beef was 10.02 at the initial

sampling. The control beef shows an almost straight line decrease in the tyrosine-tryptophane index from the first day of storage (TT = 10.13) to the 11th day (TT = 8.42). After the 11th day of storage, the decrease was gradual for the remainder of the storage period. A final value of 8.00 was noted at the end of 52 days storage at 34° F.

A value of 10.12 was found for the initial tyrosine-tryptophane index of the glycine soluble proteins of the irradiated-pasteurized beef. The values of the index were fairly constant, ranging from 10.12 to 10.00, during the first 10 days of storage after which a steady decrease occurred during the subsequent storage period. A final value of 8.12 was noted at the end of 88 days of storage.

The glycine soluble proteins were affected or modified by the irradiation-sterilization process. Prior to irradiation, the tyrosine-tryptophane index of the glycine soluble proteins of beef was 10.02 as contrasted to a value of 9.62 that was found immediately after the beef had been sterilized by an irradiation dosage of 4.8 megarads. During the first 15 days of storage, the tyrosine-tryptophane index decreased from 9.62 to 9.25. The index declined from 9.25 to 8.30 during the storage interval between 15 and 75 days of storage.

2. Glycine Soluble Amino Acids

Data of the glycine soluble amino acids (GSAA) as measured by the tyrosine-tryptophane index of control and irradiated beef are presented in Table 1 and are graphically shown in Figure 13.

A linear increase in the glycine soluble amino acids occurred in the control beef between the 5th and 18th days of storage. An index value of 4.49 was noted at the 5th day as compared to that of 6.30 obtained at the 18th day. After the 18th day of storage, a gradual but slightly irregular increase in the glycine soluble amino acid content was observed. A value of 6.63 was noted at the end of 52 days of storage.

Data of the tyrosine-tryptophane index for the pasteurized samples indicate that some changes occurred during the irradiation of the beef at dosage of 1.0 megarad. The index prior to pasteurization after irradiation. From the 5th to the 33rd day of storage, the tyrosine-tryptophane index was maintained in a narrow range between 4.18 and 4.29. After the 33rd day, there was a rapid increase in the index of the pasteurized samples. The value of 4.29 obtained at the 33rd day increased to 5.30 by the 52nd day. The index had a value of 6.25 at the end of 88 days of storage at 34° F. The index value of 6.25 obtained by the pasteurized beef

after 88 days of storage was reached by the non-irradiated beef at the end of 18 days of storage.

The glycine soluble amino acids appear to have undergone a drastic change during irradiation-sterilization at a level of 4.8 megarads. The beef prior to irradiation had a tyrosine-tryptophane index of 4.31 as compared to a value of 3.14 observed immediately after irradiation. Although the index increased at a moderate rate during the storage period, the final value of 4.28 obtained after 75 days of storage is about equal to that of 4.31 noted in the fresh beef before irradiation.

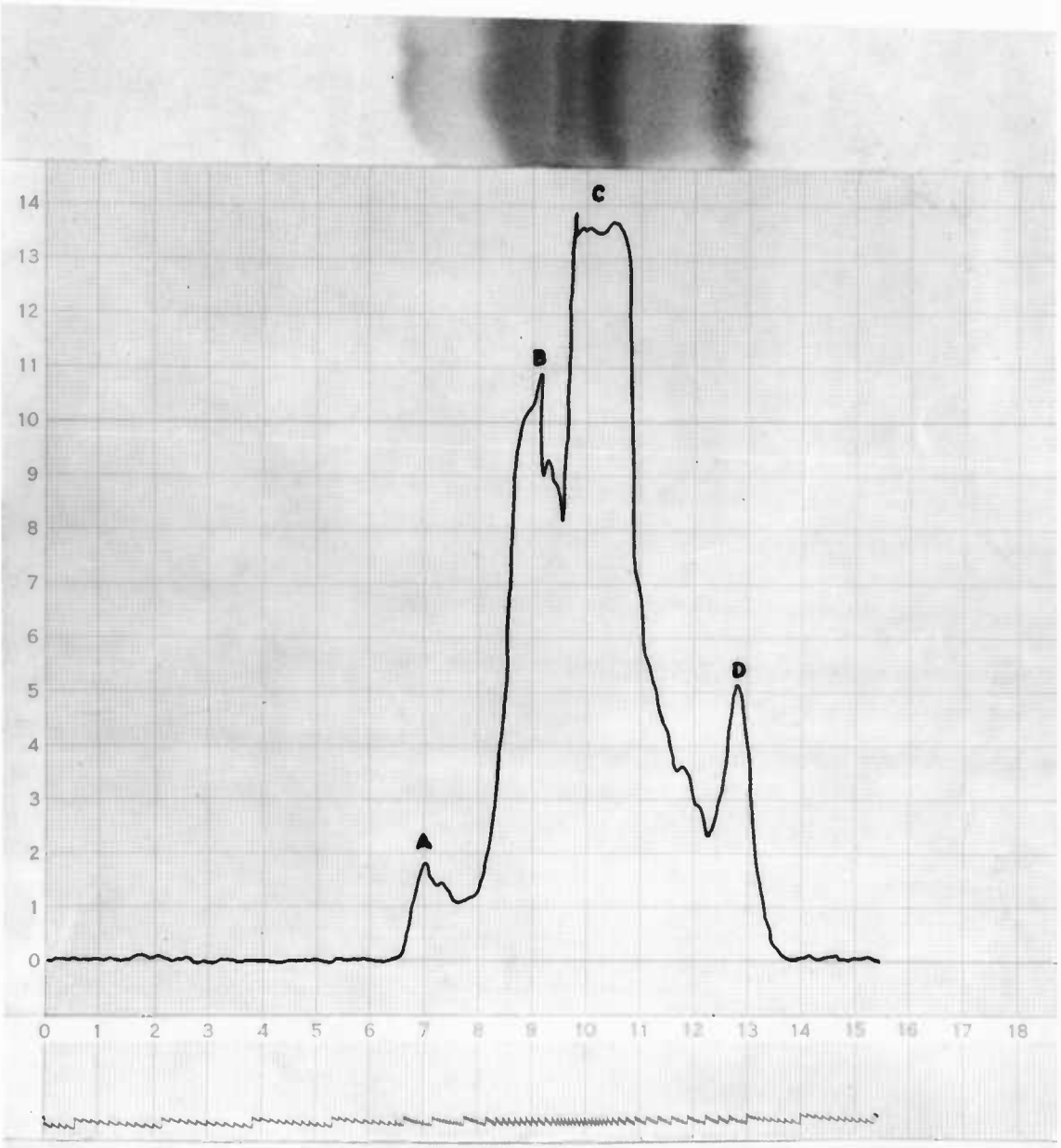

Figure 1. Paper electrophoresis pattern of glycine soluble protein of raw beef at 0 days storage at 34° F.

B-1 Veronal buffer, pH 8.6, $\Gamma/2 = 0.05$, 10 ma., 17 hours

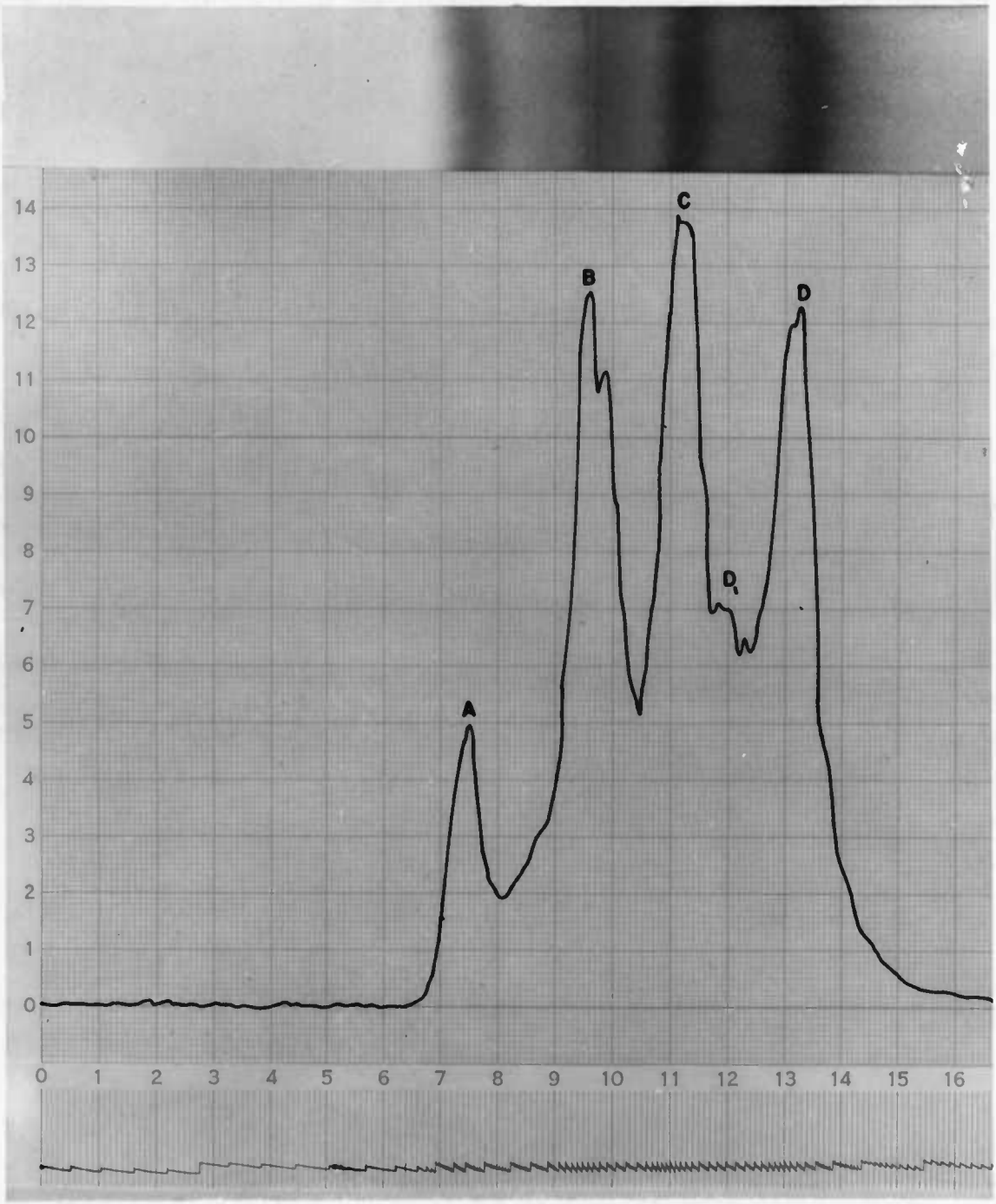

Figure 2. Paper electrophoresis pattern of glycine soluble protein of raw beef after 3 days storage at 34° F.

B-1 Veronal buffer, pH 8.6, $\Gamma/2 = 0.05$, 10 ma., 17 hours

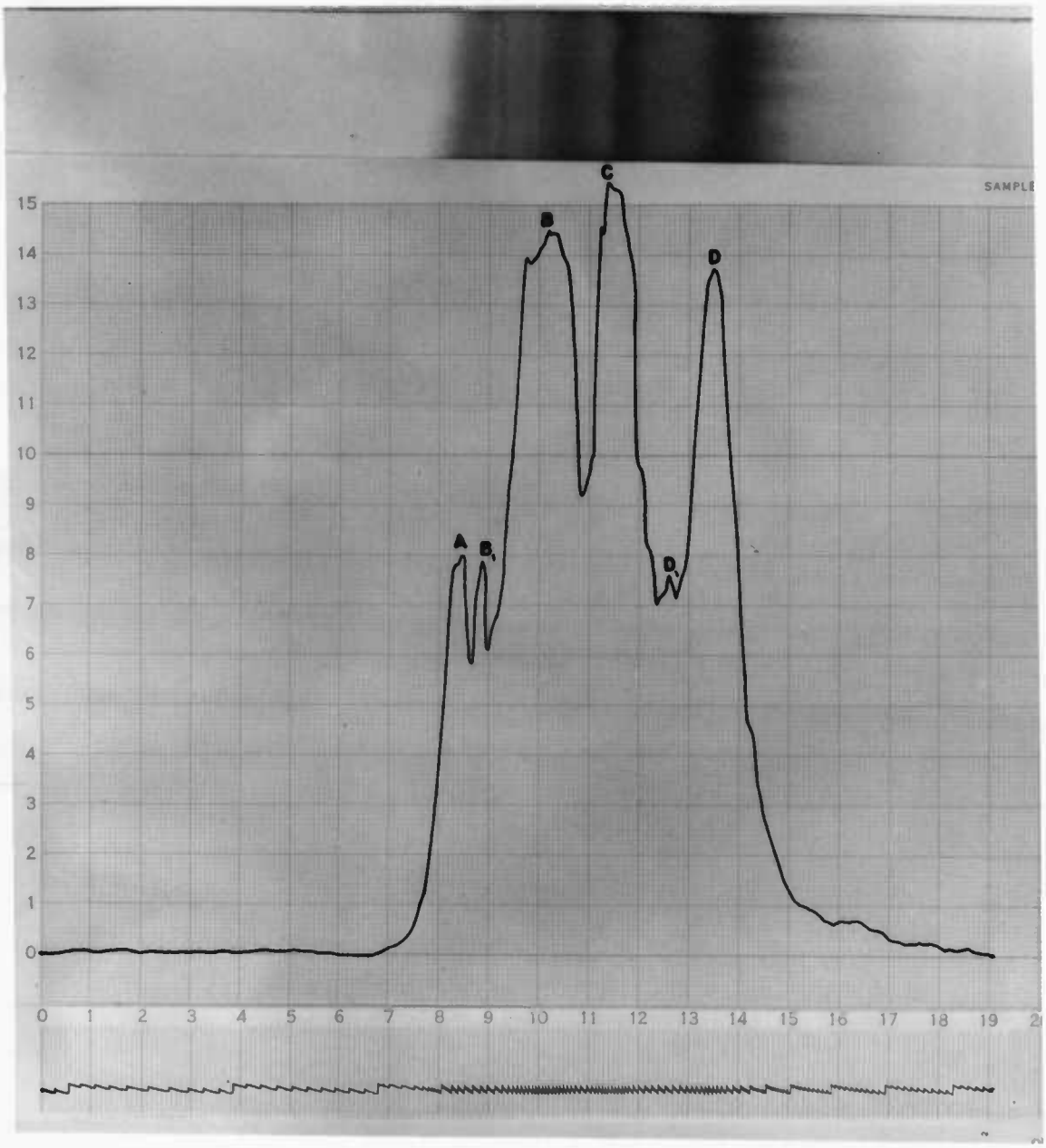

Figure 3. Paper electrophoresis pattern of glycine soluble protein of raw beef after 10 days storage at 34° F.

B-1 Veronal buffer, pH 8.6, $\Gamma/2$ = 0.05, 10 ma., 17 hours

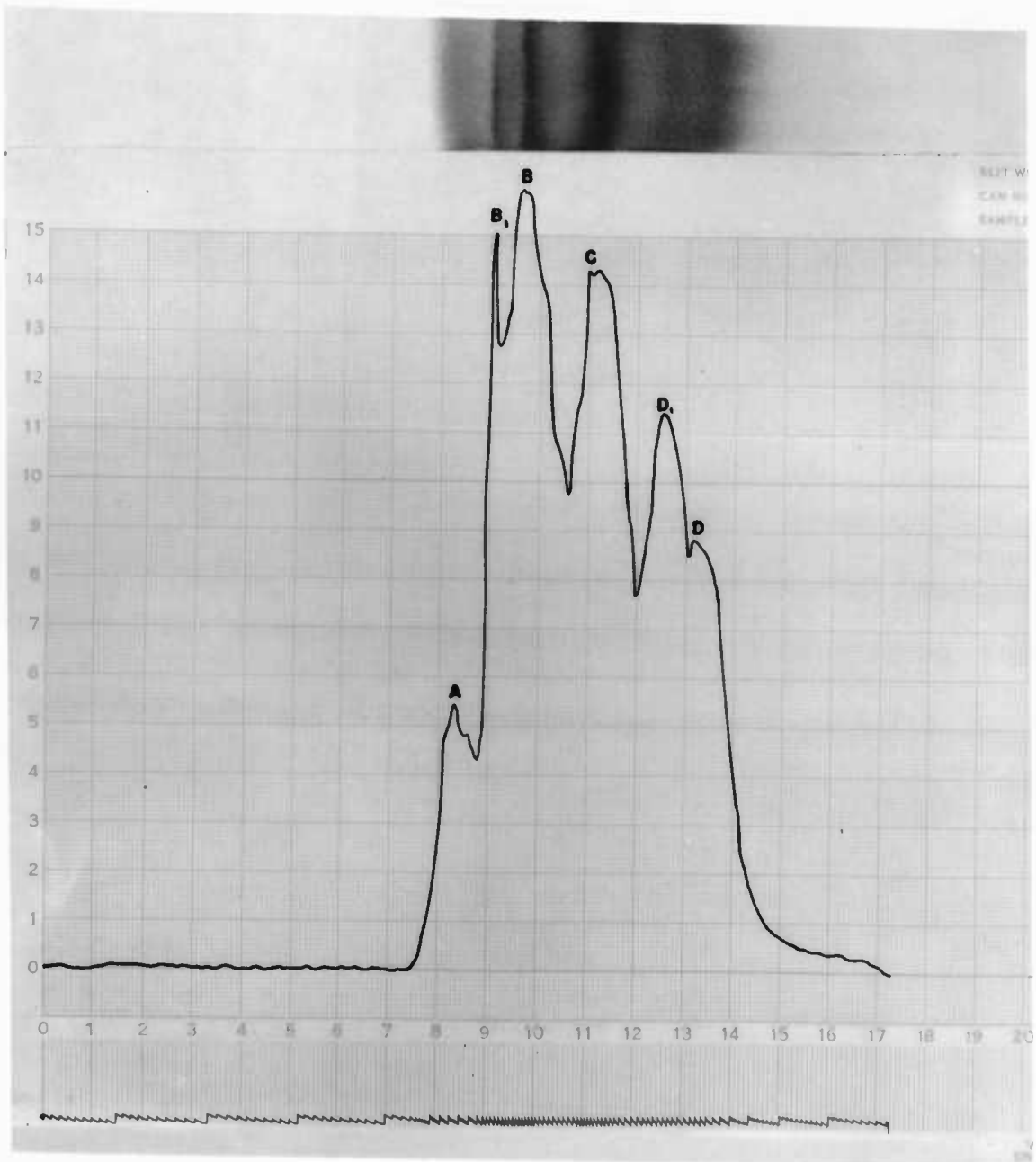

Figure 4. Paper electrophoresis pattern of glycine soluble protein of raw beef after 18 days storage at 34° F.

B-1 Veronal buffer, pH 8.6, $\Gamma/2$ = 0.05, 10 ma., 17 hours

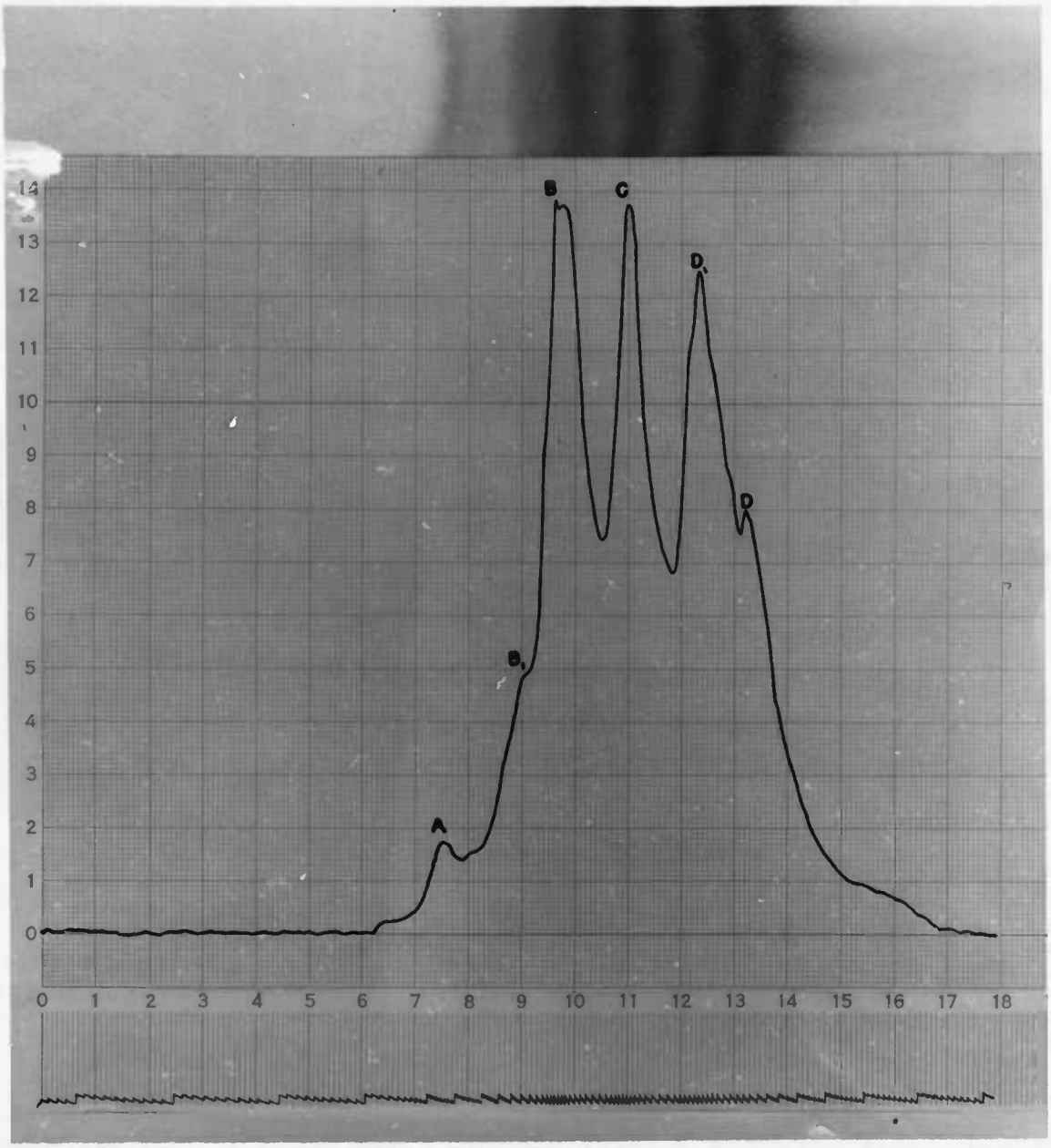

Figure 5. Paper electrophoresis pattern of glycine soluble protein beef after 49 days storage at 34° F.

B-1 Veronal buffer, pH 8.6, Γ/2 = 0.05, 10 ma., 17 hours

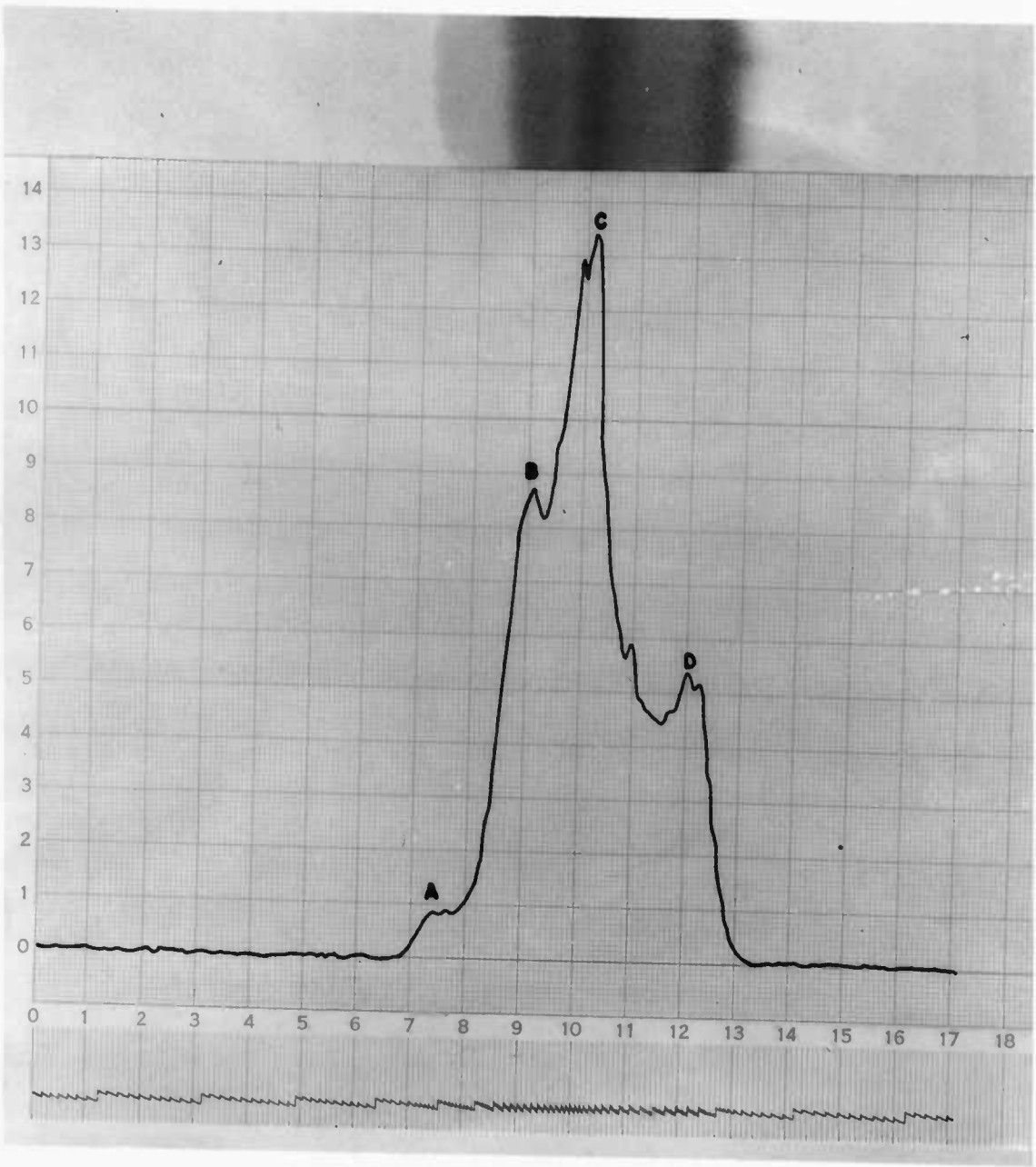

Figure 6. Paper electrophoresis pattern of glycine soluble protein of raw beef after 1 day storage at 34° F.
(control for irradiated-pasteurized samples)
B-1 Veronal buffer, pH 8.6 Γ/2 = 0.05, 10 ma., 17 hours

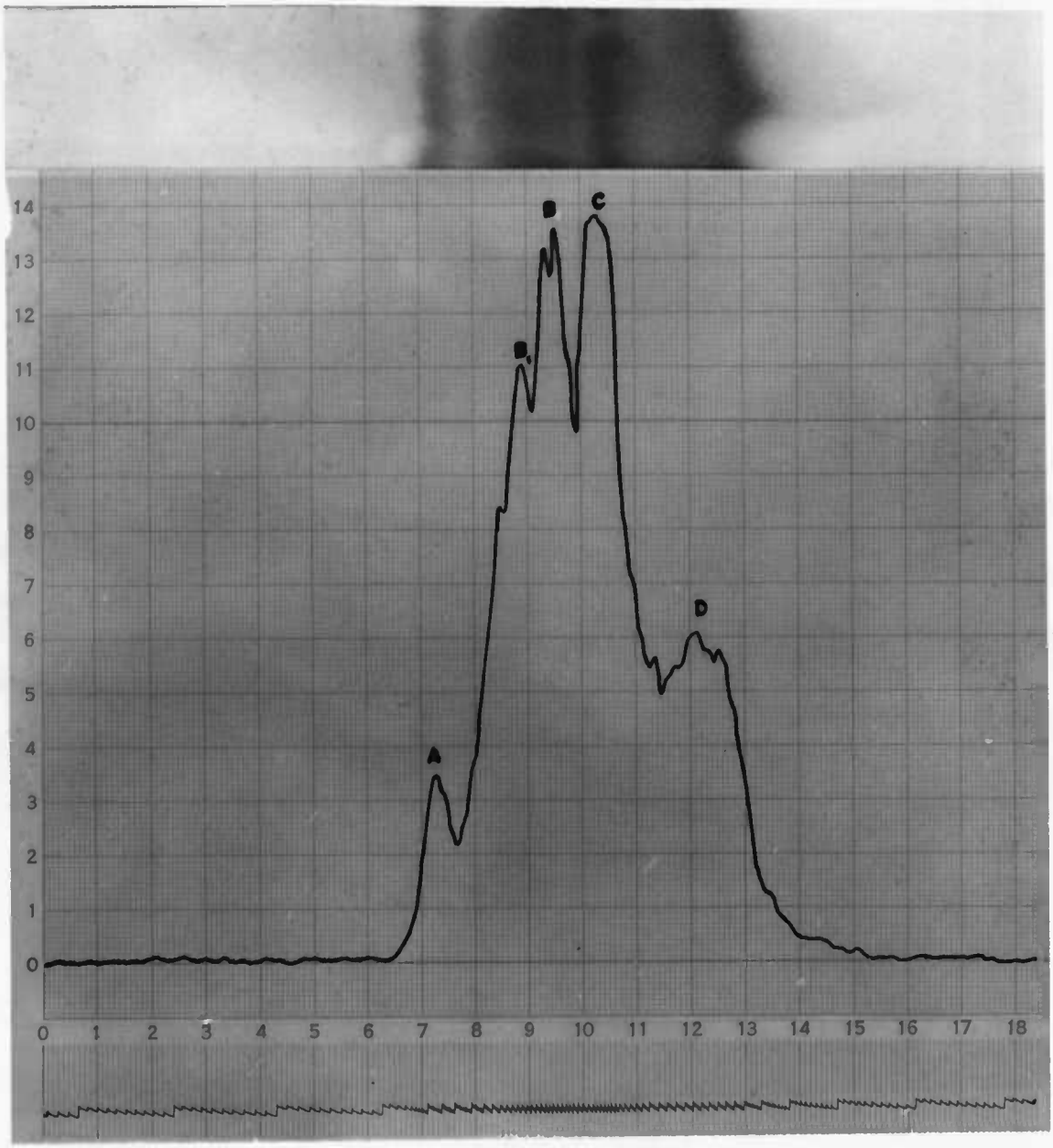

Figure 7. Paper electrophoresis pattern of glycine soluble protein of irradiated-pasteurized beef after 9 days storage at 34° F. B-1 Veronal buffer, pH 8.6, $\Gamma/2$ = 0.05, 10 ma., 17 hours

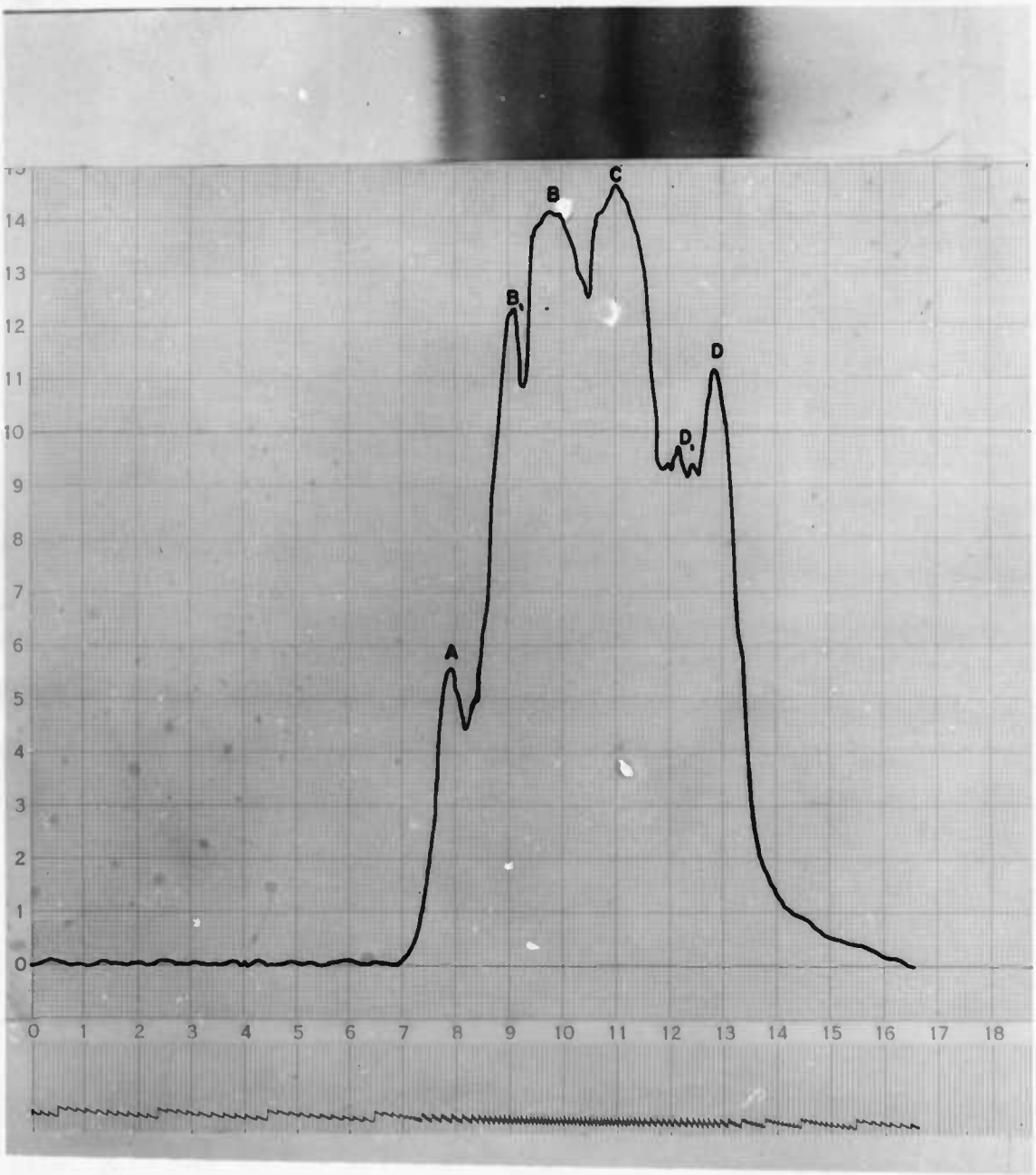

Figure 8. Paper electrophoresis pattern of glycine soluble protein of irradiated-pasteurized beef after 12 days storage at 34° F. B-1 Veronal buffer, pH 8.6, $\Gamma/2$ = 0.05, 10 ma., 17 hours

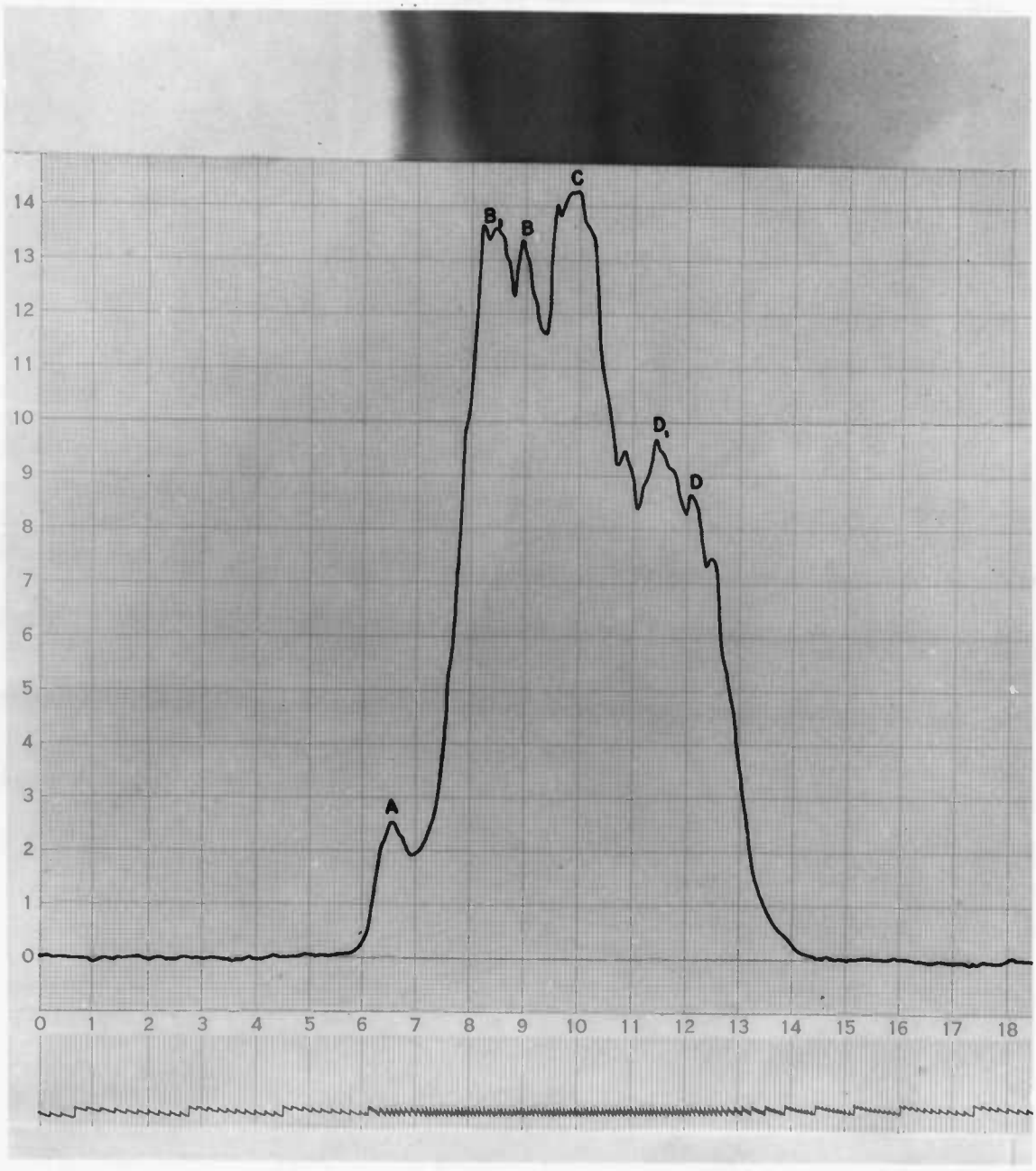

Figure 9. Paper electrophoresis pattern of glycine soluble protein of irradiated-pasteurized beef after 88 days storage at 34° F. B-1 Veronal buffer, pH 8.6, $\Gamma/2$ = 0.05, 10 ma., 17 hours

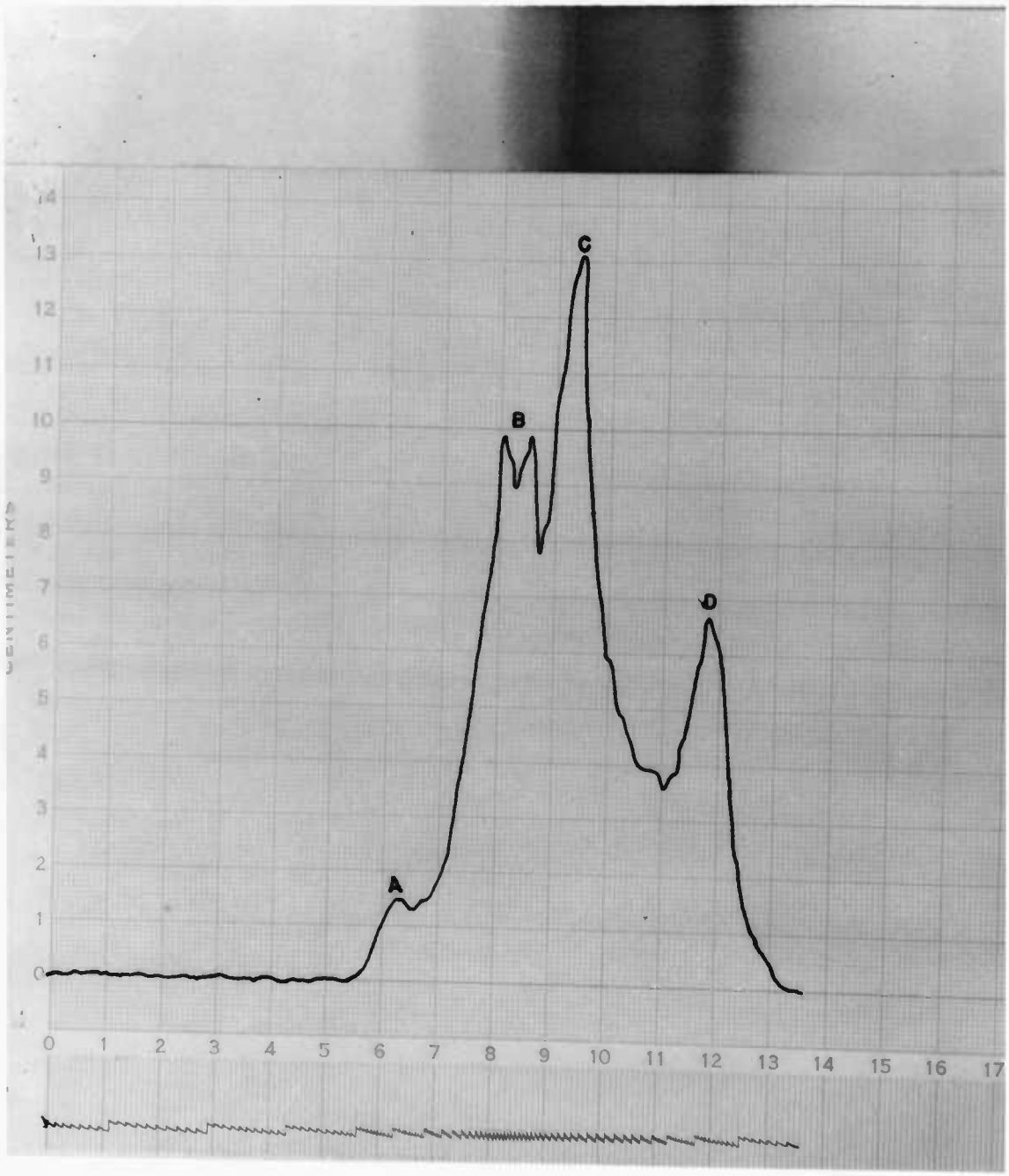

Figure 10. Paper electrophoresis pattern of glycine soluble protein of irradiated-sterilized beef immediately after irradiation. B-1 Veronal buffer, pH 8.6, $\Gamma/2 = 0.05$, 10 ma., 17 hours

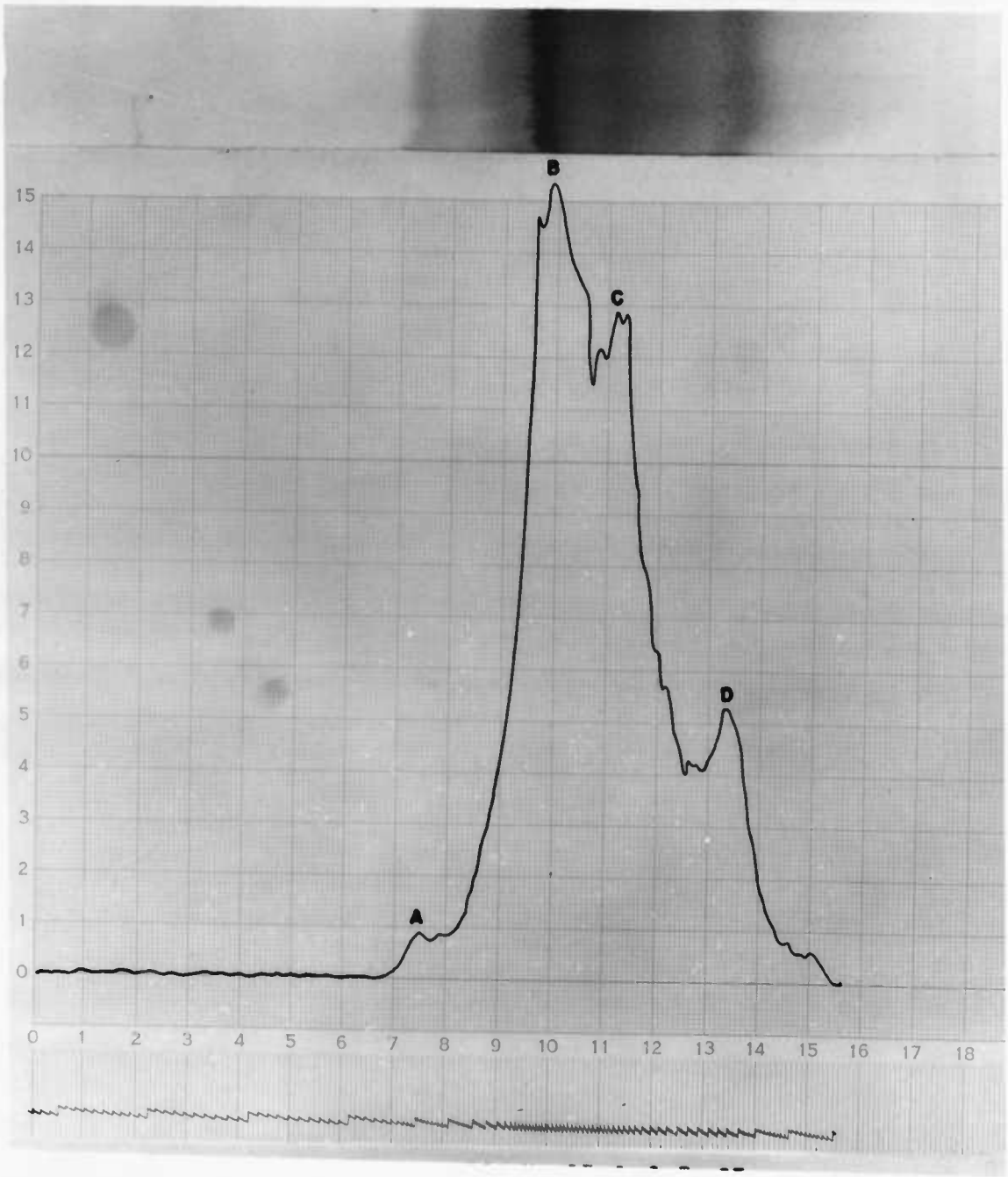

Figure 11. Paper electrophoresis pattern of glycine soluble protein of irradiated-sterilized beef after 75 days storage at 34° F. B-1 Veronal buffer, pH 8.6, $\Gamma/2 = 0.05$, 10 ma., 17 hours

TABLE 1

Tyrosine-Tryptophane Index of Control, Irradiated-Pasteurized and Irradiated-Sterilized Beef Samples Stored at 34° F.

Storage Days	Glycine Soluble Proteins Optical Density 279 mμ			Glycine Soluble Amino Acids Optical Density 279 mμ		
	Control	Pasteurized *	Sterilized **	Control	Pasteurized *	Sterilized **
0	10.02	10.12	10.02	4.31	4.36	4.31
(after irradiation)		10.05	9.62		4.02	3.14
3	9.76	10.08	-	4.49	4.15	-
6	9.52	10.00	-	4.55	4.24	-
9	9.22	10.00	-	4.80	4.26	-
12	8.40	9.80	-	5.32	4.25	-
15	8.32	9.77	9.25	5.90	4.29	3.29
18	8.21	9.52	-	6.25	4.25	-
25	8.19	9.23	-	6.32	4.20	-
35	8.18	9.06	9.17	6.48	4.52	3.78
45	8.05	8.81	-	6.57	4.90	-
52	8.00	8.42	-	6.63	5.30	-
55	-	8.42	8.90	-	5.47	4.06
75	-	8.25	8.80	-	6.03	4.28
88	-	8.12	-	-	6.25	-

* 1.0 megarad

** 4.8 megarads

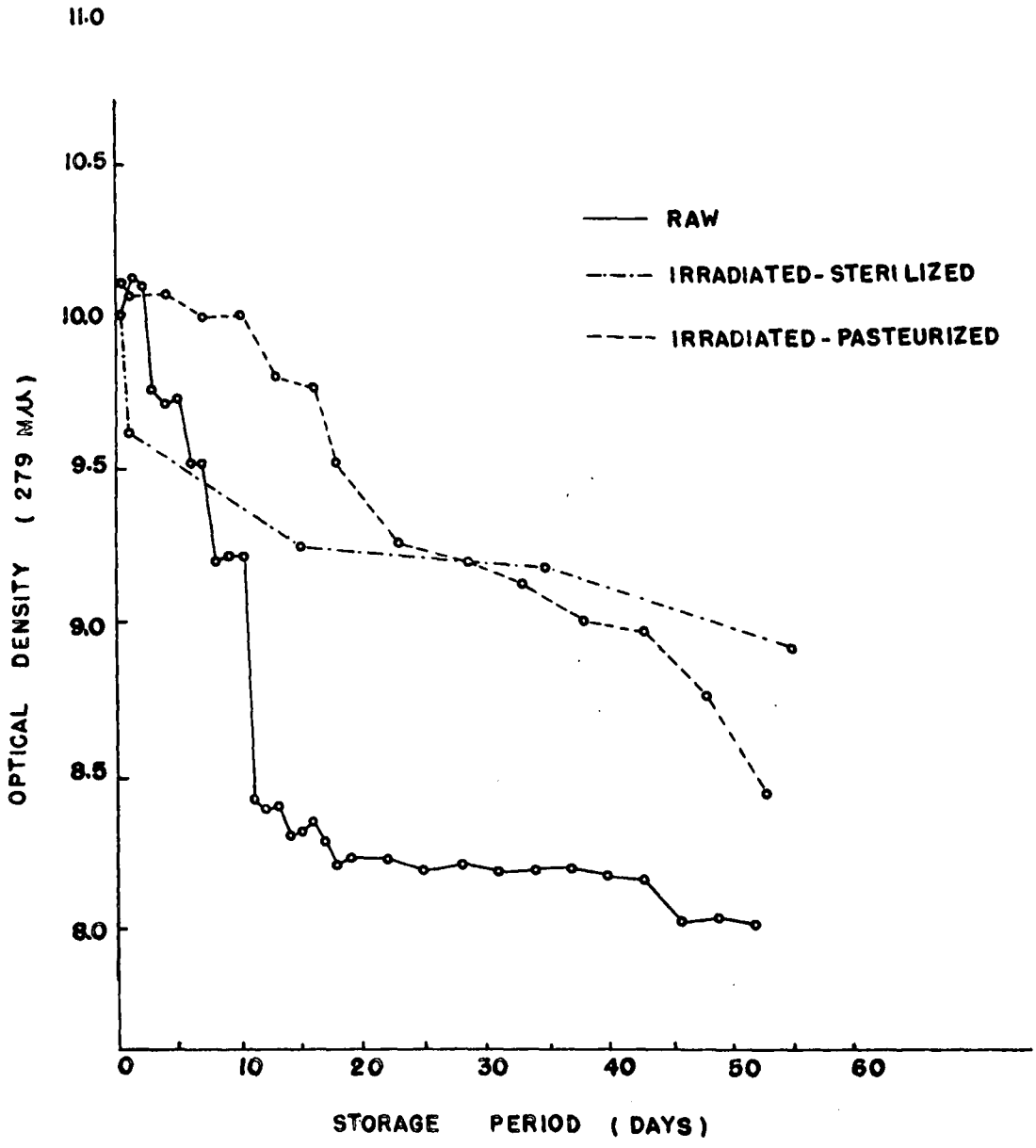

Figure 12. Tyrosine-tryptophane index of glycine soluble protein of beef stored at 34° F.

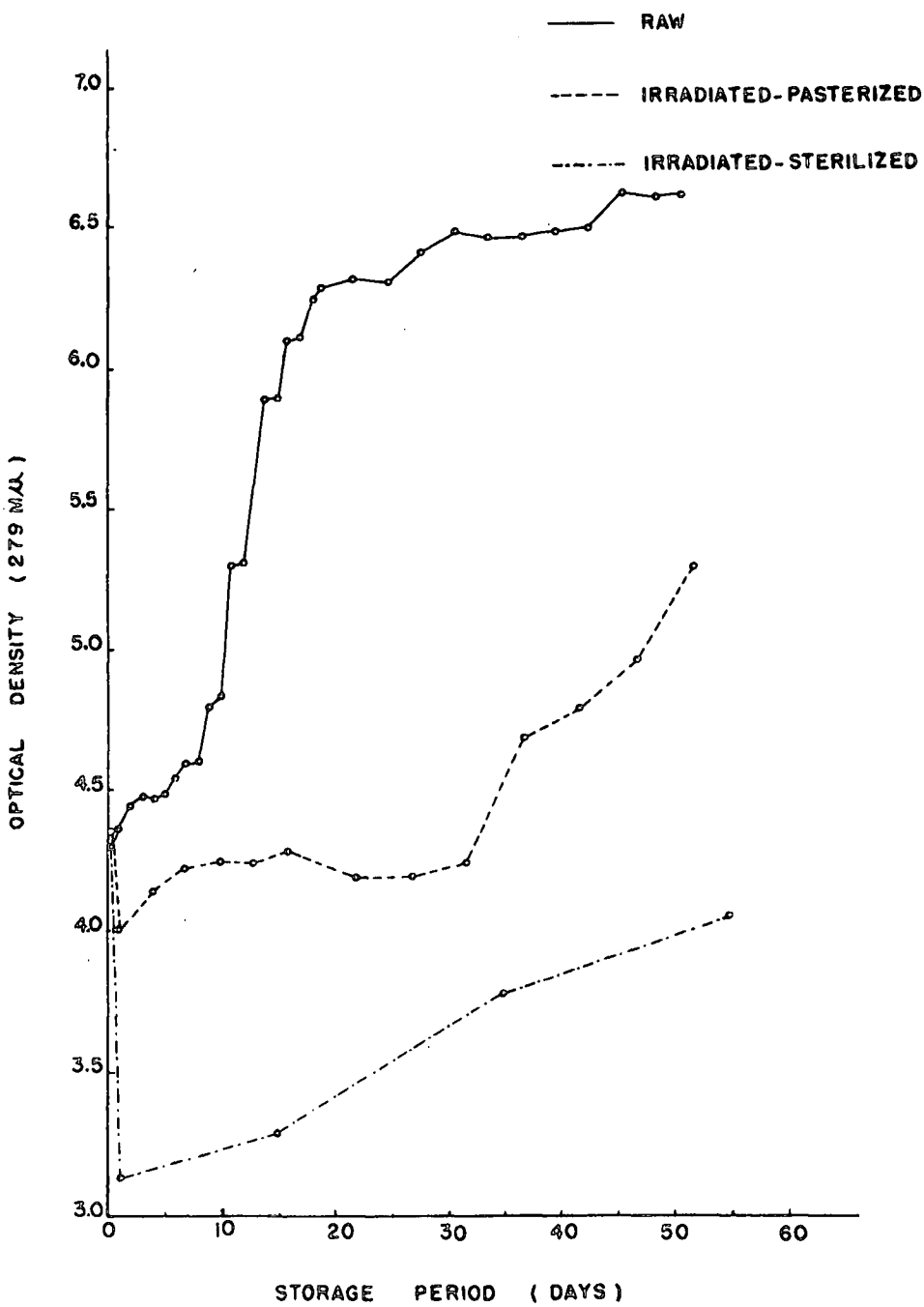

Figure 13. Tyrosine-tryptophane index of glycine soluble amino acids of beef stored at 34° F.

DISCUSSION

In the discussion of the electrophoretic patterns of the control and irradiated beef samples, the experimental data will be interpreted mostly on the basis of the reproducible appearance of new protide components. Gross changes in the four major protein fractions will also be discussed. The electrophoretic patterns and the tyrosine-tryptophane index data of the control samples are considered in the discussion as being representative of the natural processes occurring during the regular aging and storage of beef. The changes observed in the treated samples may be due to several and/or a combination of factors which will be considered in the following discussion.

In comparing the results of the non-irradiated beef with those of the irradiated samples, large differences are noted in the electrophoretic behavior of the glycine soluble nitrogenous material. The data also suggest that the extent of these differences are related to the dosage level of irradiation employed.

The electrophoretic diagrams and the tyrosine-tryptophane index data show that the control beef was subjected to proteolysis shortly after the storage period was initiated. The proteolytic activity appeared to reach its maximum rate during the interval between the 2nd and 10th days of storage. As noted in diagrams,

Figures 2 and 3, two new electrophoretic protide fractions, B_1 and D_1, appeared during this storage interval. A linear decrease in the tyrosine and tryptophane content of the glycine soluble proteins accompanied by a moderate increase in these amino acids of the glycine soluble amino acids was also observed during this storage period. After the 10th day of storage, the proteolytic activity appeared to occur at a slower rate, although there was a linear increase in the tyrosine-tryptophane index of the glycine soluble amino acids between the 10th and 18th days of storage. The increase in the index of the glycine soluble amino acids during this time would indicate that the protide fractions were being degraded at the highest rate attained any time during the storage period. Although proteolysis continued to occur between the 18th and 49th days of storage, the rate was considerably decreased.

The beef pasteurized by an irradiation dosage of 1.0 megarad showed much less proteolytic activity during the first 10 days of storage than did the control beef. In the pasteurized samples, the first new electrophoretic protide component did not appear until after 9 days of storage as compared to 3 days for that of the control. The second protide component appeared in the pasteurized samples after 12 days of storage as contrasted to 10 days for that occurring in the control samples. Although two new electrophoretic protide components (B_1 and D_1) emerged

in both the control and pasteurized samples during the storage time just mentioned, the sequence of appearance of these fractions differed according to the treatment. In the pasteurized beef, fraction B_1 appeared 3 days before the emergence of D_1. Conversely, D_1 appeared 7 days prior to B_1 in the control samples.

The data of the tyrosine-tryptophane index indicate that the proteolytic rate of the pasteurized samples was considerably lower than that encountered in the control samples. Furthermore, the increase of tyrosine and tryptophane in the glycine soluble amino acids and their corresponding decrease in the glycine soluble proteins was maintained at a more gradual rate than was noted for the control samples. Due to the similarities in the electrophoretic pattern of the control beef aged for 18 days and that of the pasteurized beef stored for 88 days, it appears reasonable to assume that the irradiation-pasteurization treatment inhibited protein degradation to a considerable extent.

The electrophoretic data of the samples sterilized by an irradiation dosage of 4.8 megarads indicate that this treatment drastically reduced proteolysis during the 75 day storage period. Although the sterilized samples did not form any new electrophoretic protide components during the storage period, some changes in the relative areas of the 4 protide components indicates that some modification occurred within the original components. The sterilized samples, immediately after irradiation, had a lower

tyrosine-tryptophane level in the glycine soluble proteins than did either the control or pasteurized samples. However, the control samples after 15 days of storage and the pasteurized beef after 35 days storage, exhibited lower glycine soluble protein tyrosine-tryptophane levels than did the sterilized samples.

The low glycine soluble amino acid index recorded immediately after sterilization indicates that an irradiation level of 4.8 megarads caused a drastic alteration in the enzymatic proteolytic activity and/or in the protein components. After 75 days of storage, the sterilized samples reached a tyrosine-tryptophane index value in the glycine soluble amino acids equal to that found in the fresh beef prior to irradiation and storage.

The observation that irradiation caused an initial decrease in the glycine soluble proteins of the sterilized samples can be explained by a number of factors or conditions reported in the literature pertaining to the effects of irradiation. Zender et al. (69, p.390) reported that irradiation causes an insolubilization of the glycine soluble proteins by a denaturation effect. Such effects as polymerization of protein fragments (41, p. 424) and partial inactivation of proteolytic enzymes (48, p. 99-109) could also be involved in causing the above noted decrease.

Although Zender et al. (69, p. 390) stated that the glycine

soluble amino acids were not affected by irradiation, data plotted in Figure 13 show that irradiation undoubtedly caused a significant decrease in the initial levels of these amino acids. This finding can be supported by indirect evidence recorded in the literature which states that irradiation causes deamination of amino acids (30, p. 7-10) and inactivates enzymes by destroying some of the side chain groups of the prosthetic portion of the enzymes which are essential for their activity (14, p. 1367). Moreover, free radicals formed during irradiation may modify the substrate conditions to inhibit proteolysis. Chemically, the free radicals are extremely active and they may combine with molecules, atoms or other nearby free radicals to form new compounds or re-form the original compound (19, p. 156-158). In addition, irradiation has been reported to cause an increase in the pH of meat (7, p. 64-67) which may also delay proteolysis because the proteolytic enzymes require acid conditions for optimal activity (23, p. 700-702). Furthermore, it is evident that the greater the dosage of irradiation, the greater the decrease in the tyrosine-tryptophane index of the glycine soluble amino acids.

In Figure 13, the curve for the tyrosine-tryptophane index of the glycine soluble amino acids of the pasteurized samples remains fairly constant during the first 33 days of storage after

which the index shows a fairly rapid rise in the tyrosine-tryptophane levels. The time that the index remained stable may represent a lag phase of microbial growth for those organisms not destroyed by pasteurization. After 30 or more storage days, conditions may have become favorable for increased bacterial growth and activity which in turn might account for some of the increase in the tyrosine-tryptophane index in the latter part of this storage period.

SUMMARY AND CONCLUSIONS

The effect of the catheptic enzymes in the proteolysis of beef was studied. In order to minimize or eliminate the microbiological effects, beef samples were irradiated at a pasteurization level of 1.0 megarads or at a sterilization dosage of 4.8 megarads, respectively. The samples were stored at 34° F. for varying lengths of time, 52 to 88 days, depending on the treatment.

The samples were analyzed at systematic intervals to determine the nature of protein degradation occurring during the storage period. Changes in the protein components were determined by paper electrophoresis technique while differences in the protide concentrations were measured by an index based on the solubility of tyrosine-tryptophane in glycine and trichloroacetic acid.

Conclusions

1. The initial electrophoretic patterns of the control, irradiated-pasteurized and irradiated sterilized beef samples were very similar, each showing the same four distinct protein components (fractions A, B, C and D).
2. The control beef showed a new electrophoretic protide fraction (D_1) after 3 days of storage and another new component (B_1) after 10 days. After 9 days of storage, fraction B_1 was the first new electrophoretic component to appear in the pasteurized samples followed by the emergence of D_1 after 12 days of

storage. The sterilized samples did not show any new electrophoretic fractions during 75 days of storage.

3. The electrophoretic pattern of the irradiated pasteurized beef after 88 days of storage resembled that of the control stored for 18 days.

4. The irradiation-sterilization process caused a large decrease in the initial tyrosine-tryptophane index of the glycine soluble amino acids while only a slight reduction was noted in the pasteurized samples. Sterilization also caused an apparent decrease in the initial levels of the glycine soluble proteins.

5. The control beef showed the greatest amount of changes in the tyrosine-tryptophane index of the glycine soluble proteins and amino acids, while moderate changes were noted in the pasteurized samples. The sterilized beef showed the least change.

6. The level of the glycine soluble amino acids remained fairly constant for 33 days in the pasteurized samples then increased at a moderate rate during the remainder of the storage period. The tyrosine-tryptophane index of the pasteurized samples was 6.25 after 88 days of storage, equaling the value obtained by the control samples after 18 days of storage. The sterilized samples reached a final value of 4.28 which was similar to that obtained prior to sterilization.

7. The rate of proteolysis was inversely proportional to

irradiation dosage. The sterilized samples showed the lowest degree of proteolytic activity.

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